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Review

MC1R and the response of melanocytes to ultraviolet radiation

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Abstract

The constitutive color of our skin plays a dramatic role in our photoprotection from solar ultraviolet radiation (UVR) that reaches the Earth and in minimizing DNA damage that gives rise to skin cancer. More than 120 genes have been identified and shown to regulate pigmentation, one of the key genes being melanocortin 1 receptor (*MC1R*) that encodes the melanocortin 1 receptor (MC1R), a seven-transmembrane G protein-coupled receptor expressed on the surface of melanocytes. Modulation of MC1R function regulates melanin synthesis by melanocytes qualitatively and quantitatively. The MC1R is regulated by the physiological agonists α-melanocyte-stimulating hormone (αMSH) and adrenocorticotropic hormone (ACTH), and antagonist agouti signaling protein (ASP). Activation of the MC1R by binding of an agonist stimulates the synthesis of eumelanin primarily via activation of adenylate cyclase. The significance of cutaneous pigmentation lies in the photoprotective effect of melanin, particularly eumelanin, against sun-induced carcinogenesis. Epidermal melanocytes and keratinocytes respond to UVR by increasing their expression of αMSH and ACTH, which up-regulate the expression of MC1R, and consequently enhance the response of melanocytes to melanocortins. Constitutive skin pigmentation dramatically affects the incidence of skin cancer. The pigmentary phenotype characterized by red hair, fair complexion, inability to tan and tendency to freckle is an independent risk factor for all skin cancers, including melanoma. The *MC1R* gene is highly polymorphic in human populations, and allelic variation at this locus accounts, to a large extent, for the variation in pigmentary phenotypes and skin phototypes (SPT) in humans. Several allelic variants of the *MC1R* gene are associated with the red hair and fair skin (RHC) phenotype, and carrying one of these variants is thought to diminish the ability of the epidermis to respond to DNA damage elicited by UVR. The *MC1R* gene is considered a melanoma susceptibility gene, and its significance in determining the risk for skin cancer is of tremendous interest.

Keywords: Melanocortin 1 receptor (MC1R); Ultraviolet radiation (UVR); αMSH; Pigmentation; Melanocyte

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Contents

1. Solar ultraviolet radiation

The main etiological factor for skin cancer (basal and squamous cell carcinoma and melanoma) is sun exposure. Solar radiation that reaches the Earth is a complex physical entity that consists of a continuous spectrum of electromagnetic radiation that can be divided into three major regions: infrared (700–2000 nm) and visible (400–700 nm), which contribute equally to about 87% of total sunlight energy, and solar ultraviolet radiation (UVR) (200–400 nm), which comprises the remaining 13% [1].

In 1801, Johann W. Ritter, a German physicist, inspired by Herschel's [2] prior discovery of infrared radiation, conducted experiments with silver salts and a prism [3]. He projected a beam of sunlight through the prism, which split the beam into the colors of the spectrum. He then exposed the salts to each color to see the outcome. The red caused a small change while the deep violet darkened the silver salts. Ritter placed salts in the lightless area just beyond the violet and it darkened with smoke. This was evidence of another waveform just barely higher than the violet of visible light. It is now known as ultraviolet (from the latin word "ultra" meaning beyond) or UVR.

The region representing UVR, the most significant region of sunlight with respect to skin cancer, lies in the range of 200–400 nm, just above visible light at 400–700 nm [4]. UVR can be further subdivided into UVA (320–400 nm), UVB (290–320 nm with a peak at 305 nm) and UVC (200–290 nm with a peak at 254 nm) [5–8]. The UVC contribution to the development of skin cancers is considered negligible, since it is prevented from reaching the surface of the Earth by the

atmospheric ozone layer that blocks UVR below about 300 nm. Unlike UVC, UVA and UVB both reach the Earth's surface in amounts sufficient to cause harmful biological effects on the skin. It is estimated that 5% of solar UVR is UVB [9].

The spectral characteristics of solar radiations undergo shifts while traveling from the sun to the Earth's surface. Absorption in the spectral region below 85 nm is mainly due to O, O_2, N and N_2. The absorption above the stratosphere for the 85–200 nm is due to O_2. When solar rays reach the stratospheric layer, ozone O_3 absorbs the remaining solar UVR in the 200–288 nm wavelength range. Thus, essentially solar UVR shorter than 288 nm is absorbed by the Earth's atmosphere [10].

UV exposure varies continuously with the time of the day, the season of the year, the zenith angle of the sun, the cloud cover and the changes in reflecting surfaces. These uncertainties make the assessment of solar UVR impinging on human skin very difficult. For example, the total radiation reflected from grass is about 3%, 20–30% from sand, about 5% from water and up to 90% from snow [10]. As the sun falls lower in the sky, the path length of the sun's UV rays through the atmosphere increases and as a consequence the intensity of UV reaching the Earth's surface decreases at all wavelengths, particularly those shorter than 320 nm. The sun's UV rays are strongest in the 4-h period around local noon when 50–60% of a summer's day UV is received. Exposure to the sun before 11 a.m. and again after 3 p.m. until the end of the day avoids most of the ambient available UV [11].

Since pure water is a very weak absorber of UVR, clouds, which are composed of either liquid or ice droplets, attenuate UV primarily by scattering. Clouds reduce UV intensity, although not to the same extent as infrared intensity. This is because water in clouds attenuates solar infrared much more than UV and so the risk of overexposure is increased because the warning sensation of heat is diminished.

2. Ozone

For over 50 years, chlorofluorocarbons (CFCs) were thought of as miracle substances. They are stable, nonflammable, low in toxicity and inexpensive to produce. Over time, CFCs found uses as refrigerants, solvents, foam blowing agents, and in many other smaller applications. Other chlorine-containing compounds include methyl chloroform, a solvent, and carbon tetrachloride, an industrial chemical. Halons, extremely effective fire-extinguishing agents, and methyl bromide, an effective produce and soil fumigant, contain bromine. All of these compounds have atmospheric lifetimes long enough to allow them to be transported by winds into the stratosphere [12]. Because they release chlorine or bromine when they break down, they damage the protective ozone layer [13]. The ozone layer, measured in Dobson Units, is formed 100 km above the Earth's surface: $O_2 + UVC = O + O$ and then, in the stratosphere $O_2 + O = O_3$. But when CFCs are exposed to UVR, the CFC molecules release atomic chlorine: $CFCl_3$ (CFCs) + UV = 3 Cl. One chlorine atom can destroy over 100,000 ozone molecules. The net effect is to destroy ozone faster than it is naturally created $Cl + O_3 = ClO + O_2$ and $ClO + O = Cl + O_2$ (from US Environmental Protection Agency www.epa.gov).

Recognition that CFCs are contributors to stratospheric chlorine levels and that stratospheric ozone depletion is a chlorine-catalyzed process [13] has led international authorities to mandate the phase-out of CFCs in 1996. Hydrochlorofluorocarbons and hydrofluorocarbons are being developed as substitutes for CFCs. This results in a much smaller half-life and lower ozone depleting potential for these products in comparison with CFCs [14]. In addition, research has shown that ozone depletion occurs over the latitudes that include North America, Europe, Asia, and much of Africa, Australia, and South America. Thus, ozone depletion is a global issue and not just a problem at the South Pole [15].

UVC rays are the strongest, most dangerous type of UV. Little attention has been given to UVC rays in the past since they are normally filtered out by the ozone layer and do not reach the Earth. However, thinning of the ozone layer and holes in the ozone layer are causing increased concern about the potential for UVC radiation exposure [16–20]. Concerns are also rising about the biologically harmful effects of less UVB being filtered by the depleted ozone layer [15,21,22]. Krishnamurthy, in 1997 [23], showed that melanoma incidence and frequency increase with decreased ozone levels and increased UV light exposure.

3. Minimal erythemal dose (MED) and skin phototype

Careful studies of human skin [24] revealed no significant differences in the actual number of melanocytes. Instead, ethnic differences in skin color come mainly from differences in the rate at which melanosomes are produced and melanized in melanocytes, and then distributed and transferred to neighboring keratinocytes [25].

The concept of "sun reactive" skin typing was created in 1975 for a specific need: to be able to classify persons with "lightly pigmented skin" in order to select the initial correct doses of UVA for the application of the newly developed technique to treat psoriasis using psoralen and UVA [26,27]. A simple classification with only four skin types was initially proposed based on the patient response to an initial sun exposure of 3 MEDs. Later, a complete classification with six skin types was established [27].

In phototesting, a number of skin sites (usually 1 cm^2) of normal skin are exposed to increasing doses of UVR and are visually examined for erythema 24 h later [28] to determine the amount or dose of simulated sunlight that they can receive before their skin begins to turn slightly red (Fig. 1). This is called their MED. The MED varies from person to person depending on their skin type. Fairer people tend to go red more quickly than darker people who have more pigment in their skin. An MED is defined as the lowest exposure that produces a minimally perceptible redness [29]. The assessment of MED is to have an objective determination of the sensitivity of the skin to UV.

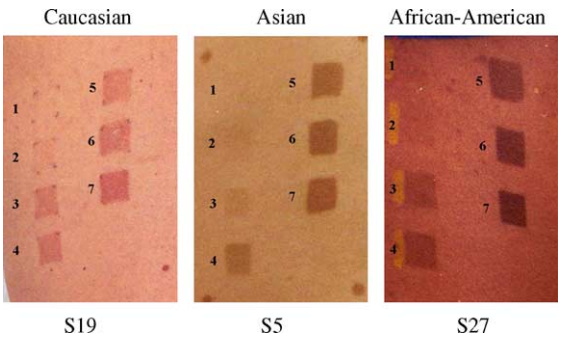

Fig. 1. Skin erythema and pigmentation at day 8 after UVB/UVB irradiation at various doses. Back skin of one subject for each ethnic group studied for MED determination is shown: S19 Caucasian, S5 Asian, S27 African–American. Numbers 1–7 correspond to the following UV doses (in J/m^2): S19, 1 = 197, 2 = 380, 3 = 498, 4 = 552, 5 = 789, 6 = 1104, 7 = 1577; S5, 1 = 150, 2 = 210, 3 = 300, 4 = 420, 5 = 600, 6 = 840, 7 = 1200; S27, 1 = 406, 2 = 565, 3 = 809, 4 = 1120, 5 = 16326 = 2311, 7 = 3323. MEDs were determined as 295 J/m^2 for S19, 200 J/m^2 for S5 and 765 J/m^2 for S27.

The ability to adjust melanization of epidermal cells after sunlight exposure reflects the individual's melanogenic potential, which led to the concept of facultative and constitutive skin color [30]. Constitutive skin color designates a genetically determined level of cutaneous melanin, in the absence of acquired exogenous or endogenous influences. Facultative pigmentation, on the other hand, designates an induced level of increased epidermal melanin content as a result of solar radiation, hormones or other environmental factors. This provides clear separation of two groups of skin phototypes (SPT): sun-sensitive (SPTs I–III who burn and tan with difficulty or not at all) and sun-tolerant (SPTs IV–VI who tan easily and do not burn). In most cases, phototypes show a strong correlation with MEDs [31], except for studies on Korean brown skin [32].

It was recommended that the SPT should be in the medical records of children and adults and that sun-sensitive subjects should be told about their SPT and given advice regarding lifelong sun protection to avoid dermatoheliosis and skin cancer [33].

4. Photoprotective role of melanin

Melanin serves as a photoprotective agent against the damaging effects of UVR, as evidenced by an inverse correlation between the melanin content of human skin and the incidence of skin carcinomas and melanomas induced by UVR. In the US, rates of basal and squamous cell carcinomas are 50 times higher in Caucasians than in African–Americans [34], who show a 13-fold lower incidence of melanoma [35].

The exact chemical structures of the two types of melanin are still unknown, probably due to complications of copolymerization and numerous postpolymerization modifications [36,37].

The photoprotective properties of melanin in human skin have been well documented [37]. It absorbs both UV and visible light. The absorption increases linearly in the range of 720–620 nm and then exponentially toward shorter wavelengths (300–600 nm) [38]. Ultrastructural studies revealed that eumelanosomes that are

generally produced in dark skin remain intact in the epidermis after UV exposure whereas in fair skin, no intact melanosomes can be detected there [39,40].

5. The melanocortin receptors

One way to control skin pigmentation was elucidated at the beginning of the 1990s with the molecular characterization of the melanocyte-stimulating hormone receptor (MSHR) now named melanocortin 1-receptor (MC1R) and its antagonist agouti signal protein (ASP) [41,42]. It has been known for many years that two loci, *extension* and *agouti*, were involved in the qualitative (eu- and phaeomelanin) and quantitative regulation of mammalian pigmentation [43,44]. ASP was known to be produced in hair follicles and to act on follicular melanocytes to inhibit eumelanin synthesis [45,46].

Prior to cloning, two melanocortin receptors, MSHR and the adrenocorticotropic hormone (ACTH) receptor, were known from classical physiological and pharmacological studies. The melanocortin system consists of the melanocortin peptides and various forms of melanocyte-stimulating hormone (α-, β- and γ-MSH) and ACTH, a family of 5 seven-transmembrane G protein-coupled melanocortin receptors, and the endogenous melanocortin antagonists ASP and agouti-related protein. In addition, two ancillary proteins, mahogany and syndecan-3, have been found that modulate the activity of the melanocortin peptides [47]. Those peptides are primarily produced in the pituitary from the cleavage of the pro-opiomelanocortin (POMC) polypeptide. The melanocortins are involved in an extraordinarily diverse number of physiological functions, including pigmentation [48–52], steroidogenesis [53,54], energy homeostasis [55–57], exocrine secretion [58,59], stress [60], sexual function [61,62], analgesia [63], inflammation [64,65], thermoregulation [66], and cardiovascular regulation [67,68]. The role of melanocortin peptides in food intake [69–72] and body weight control [73–76] has also been widely investigated.

The MCR pathway includes five known differentially expressed G protein-coupled receptors: MC1R, corticotropin receptor (ACTHR) also named MC2R, MC3R, MC4R and MC5R [41]. Although all five receptors are known to be Gs-coupled, MC3R has also been shown to function through phospholipase C-mediated hydrolysis of phosphoinositides [77]. MC1R is predominantly expressed in melanocytes, where it is known for its classic role in regulating skin and hair pigmentation in many species. MC1R has also been reported to be expressed in other tissues and cells, such as the pituitary and leukocytes [78], mast cells [79] and pro-monocytes [80], indicating putative physiological roles yet to be unveiled. Several groups have investigated the role of MC1R in inflammation [81–83]. MC3R and MC4R are found in the central nervous system but are notably absent from melanocytes [84]; both are highly expressed in the hypothalamus, where they are involved in energy homeostasis. ACTHR is expressed in the adrenal cortex and MC5R in peripheral cells such as adipocytes [85].

αMSH is a physiologic agent that controls pigmentation in mammals by inducing melanocyte differentiation and melanin production [86,87]. The first step of αMSH action is its binding to the MC1R on the plasma membrane of the melanocyte [88,89].

6. Regulation of MC1R activity by its physiological agonists and antagonist

The significance of human cutaneous pigmentation lies in its protective role against sun-induced DNA damage and photocarcinogenesis [90,91]. Total melanin content and the relative amounts of eumelanin and pheomelanin synthesized by human epidermal and follicular melanocytes are important determinants of skin and hair color, respectively [92,93]. Stimulation of MC1R by αMSH and other POMC peptides induces an increase in intracellular cAMP and the synthesis of photoprotective eumelanin. Phaeomelanin, which may contribute to skin carcinogenesis by producing free radicals in response to UVR [94], is predominantly found in individuals with red hair and fair skin, which may explain the sun sensitivity and the poor tanning ability of these individuals [95].

Injections of human subjects with melanocortins or with an αMSH synthetic analog [96,97] resulted in an increase of cutaneous pigmentation. Treatment of cultured human melanocytes by αMSH resulted in the increase of melanogenic proteins as well as an increase in cAMP [92,98,99]. Disruption of the expression of POMC peptides results in red hair because of the lack

of eumelanin, as well as adrenal insufficiency and obesity [100].

In mice, as in many other mammals, the wild-type pigmentation pattern of the fur is called agouti. Individual hairs of an agouti coat are black, with a narrow band of pheomelanin in individual hairs. Conversely, in dominant mutations of MC1R, the agouti ring of yellow pigment just below the tip is not found; this alternative synthesis of pheomelanin versus eumelanin is regulated in a paracrine manner by the agouti locus in mice [101]. A short pulse of agouti expression at days 3–5 during the hair-growth cycle [101] leads to a controlled wave of agouti protein secretion from the dermal papillae, which results in a temporary switch to phaeomelanin synthesis [102,103].

Several studies have shown that ASP, the 131 amino acid protein encoded by the *agouti* gene, acts as a competitive antagonist of the MC1R and blocks its activation by αMSH [42,104,105]. Therefore, the switch between eumelanogenesis and pheomelanogenesis involves the opposing effects of ASP and αMSH as ligands for that receptor. As previously reported, pheomelanogenesis can be stimulated further by in vitro treatment with purified recombinant ASP [106]. After treatment with ASP, the expression of genes encoding tyrosinase and other melanogenic proteins is suppressed in melanocytes, which also exhibit other physiologic features characteristic of pheomelanogenesis in vivo. In normal human melanocytes (NHM), where the number of MC1R expressed is relatively low [107], ASP completely abrogates the stimulatory effects of αMSH on melanocyte proliferation and melanogenesis by blocking the binding of αMSH to the MC1R [99].

7. Human MC1R allelic variants: link to sun sensitivity and/or melanoma susceptibility

Human pigmentation is regulated by more than 120 genes [108], and among them, the MC1R is highly polymorphic, suggesting its significance in the wide range of human pigmentation patterns [40]. Today, *MC1R* is the only gene in which variations can explain differences in normal pigmentation in humans [109]. An effect of the *MC1R* gene on the variation in human pigmentation has been hypothesized since MC1R variants causing coat color changes are known in other mammalian species [110–114]. The *MC1R* gene has been found to be highly polymorphic in Caucasian populations and specific *MC1R* gene variants such as R142H, R151C, R160W and D294H have been associated with red hair, fair skin and freckling as well as sun sensitivity in Northern European and Australian populations [115–120]. In addition, recent studies have shown a significant association between the MC1R genotype and both familial and sporadic melanoma [121–123] as well as non-melanoma skin cancer [124].

Fair skin and red hair are also associated with an increased risk of cutaneous malignant melanoma [125]. Increased risks of malignant melanoma have been shown in subjects carrying different *MC1R* gene variant alleles [121,126,127]. Carriers of the D84E variant allele were found to have an increased risk of malignant melanoma in a study reported by Valverde and co-workers [126] and by Kennedy et al. [123], but later studies by the same research group [127] and by other research groups [121,128] were not able to confirm this association. More recently, the R151C, R160W and D294H variant alleles were reported to be associated with an increased risk of malignant melanoma [121]. The association between MC1R variants and malignant melanoma suggests that the *MC1R* gene is a susceptibility gene for this type of skin malignancy [122]. The role of *MC1R* gene variants in modulating the risk of non-melanoma skin cancer, however, is largely unknown. In a small group of British patients with non-melanoma skin cancer, an overrepresentation of the D294H variant allele was found [116]. Such an overrepresentation, however, could not be confirmed in a study of patients with basal cell carcinoma [128].

Melanocytes that express the consensus sequence for MC1R have dark pigmentation [129]. More than 30 MC1R variants have been described, of which 9 have been demonstrated to be loss of function variants [130,131]. Some of these variants (V60L, I40T, R142H, R151C, R162P, R160W, and D294H) are unable to stimulate cAMP production as strongly as the wild-type receptor in response to αMSH stimulation [132–135] whereas others (e.g., V122M) demonstrate a decreased αMSH binding affinity. Three MC1R variants alleles (R151C, R160W and D294H) have been shown to be associated with the red hair and fair skin (RHC) phenotype [136], a condition that is caused by the predominant level of phaeomelanin synthesis and that can place individuals at higher risks of skin cancer [137]. RHC is characterized by fair pigmen-

tation (fair skin, red hair and freckles), and by sun sensitivity (poor tanning response and solar lentigines) [116–120,123]. In addition, seven other alleles (V60L, 86insA, D84E, R142H, I155T, 537insC and H260P) may be statistically considered full or partial RHC causing alleles, as shown by genetic associations in populations or through inheritance of phenotype in families [137]. Furthermore, MC1R variants appear to increase the penetrance of p16INK4A mutations in melanoma prone families [122,138].

8. Response of melanocytes to UVR

We previously reported that exposure of cultured primary melanocytes to UVR induced a significant reduction in MC1R mRNA level [93]. This effect disappeared within 24 h after UV irradiation at relatively low doses, but persisted in NHM irradiated with a cytotoxic dose of UVR. The reversal of the effects of low or moderate doses of UVR might be attributed to stimulation of αMSH and ACTH production in NHM [139,140], which is expected to up-regulate MC1R expression in a paracrine fashion. We proposed a model for the UVR induced melanogenesis in NHM. UVR affects NHM directly by damaging the DNA, as well as indirectly by stimulating the synthesis of a variety of epidermal factors, such as αMSH, ACTH, bFGF and ET-1 [139–144]. Many of those factors act as paracrine regulators that mediate the effects of UVR on melanogenesis, proliferation, as well as survival of melanocytes. Although these factors activate different signaling pathways, they converge on the up-regulation of MC1R mRNA level, suggesting a central role of MC1R in determining human cutaneous pigmentation. Increasing the level of MC1R mRNA is expected to enhance the response of melanocytes to αMSH and ACTH and to mediate the pigmentary response to UVR, as proposed previously in studies using mouse melanoma cells [145–147].

Binding of αMSH and ACTH to MC1R stimulates the synthesis of cAMP (Fig. 2), activates cAMP-dependent protein kinase A, and subsequently a series of downstream targets, many of which are yet to be identified [99,147]. This cascade of events leads to the stimulation of proliferation and melanogenesis of NHM [98]. The MC1R functions as a primary regulator of eumelanin synthesis in mammalian

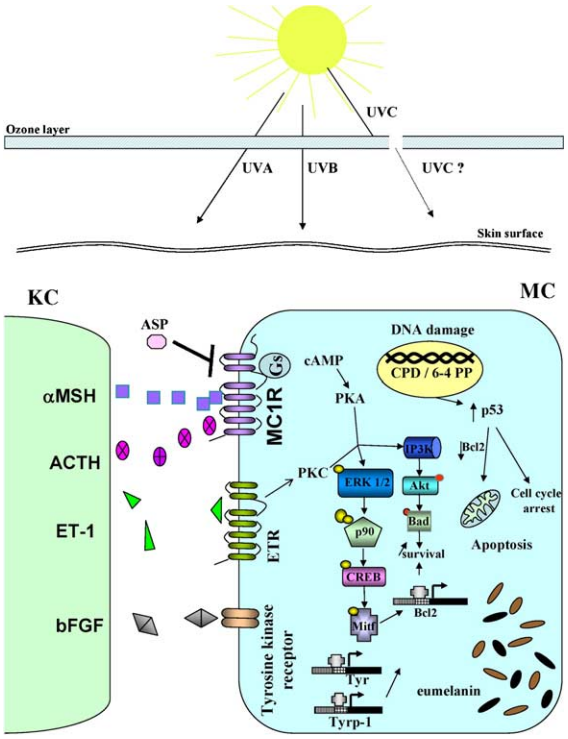

Fig. 2. Summary of the effects of UV in human skin melanocytes and keratinocytes. Exposure of human skin to UV results in the release of a variety of factors by keratinocytes, among which ACTH and MSH will bind to the MC1R and activate the cAMP/ PKA pathway. Formation of DNA photoproducts will lead to an activation of the p53 pathway, resulting in apoptosis, G1 arrest and DNA repair. Few hours after UV exposure, accumulation of p53 in response to DNA damage and reduction in Bcl2 level are observed. UV activates Akt, which in turn phosphorylates and inhibits the apoptotic effect of Bad. Upon UVR stimulation, activated CREB is expected to activate Mitf that increases melanogenesis and promotes the survival of melanocytes.

melanocytes. This has been demonstrated in mouse follicular melanocytes decades ago, and more recently in human epidermal melanocytes [93,148,149].

G protein-coupled receptors owe their name to their extensively studied interaction with heterotrimeric G proteins (composed of an α-, β- and γ-subunit), which undergo conformational changes that lead to the exchange of GDP for GTP bound to the α-subunit following receptor activation [150]. Consequently, the G subunits stimulate effector molecules, which include adenylyl and guanylyl cyclases, phosphodiesterases, phospholipase A2, phospholipase C and phosphoinositide 3-kinases, thereby activating or in-

hibiting the production of a variety of second messengers such as cAMP, cGMP, diacylglycerol, inositol (1,4,5)-trisphosphate (IP3), phosphatidyl inositol (3,4,5)-trisphosphate (PIP3), arachidonic acid and phosphatidic acid, in addition to increasing the intracellular concentration of calcium and opening or closing a variety of ion channels.

When the MC1R is activated upon ligand binding, the G-protein activates adenylyl cyclase resulting in a significant increase of intracellular cAMP. In cultured human melanocytes and in mouse melanoma cells, αMSH and ACTH up-regulate melanogenesis and dendricity [98,151]. These effects can be mimicked by pharmacological cAMP elevating agents such as forskolin, cholera toxin or isobutylmethylxanthine [152].

9. Response of MC1R to UV/αMSH with respect to the various genotypes/phenotypes

More than 30 allelic variants of the human MC1R have been identified, mainly in Northern European populations and in Australia [115,116,120,153]. As noted above, among the variants so far reported, R142H, R151C, R160W and D294H are the mutations mostly associated with red hair phenotype and reduced tanning ability [116,118,120,154]. These four MC1R variants are common in melanoma patients, and increase the risk of melanoma by more than two-fold [121]. Homozygote or compound heterozygote variant MC1R genotype carriers are generally red-haired, however, the existence of exceptions indicate that other loci are involved in the phenotype expression [120]. Red hair occurs in a significant proportion of compound heterozygote variants, and some alleles are shown to be stronger (D84E, R151C, R160W and D294H) than others (V60L, V92M and R63Q) in determining the phenotype [154].

The consequences of these variants on physiological function of the MC1R have just begun to be uncovered. It is known that R160W homozygote and R151C/D294H, R160W/D294H compound heterozygote NHM, fail to couple to cAMP activation, show impaired tyrosinase activation in response to αMSH stimulation and display a pronounced sensitivity to UVR [93]. Similar failures in activating cAMP formation were also demonstrated in heterologous expression systems [132,133]. The significance of the MC1R in cutaneous responses to UVR is emphasized by the exaggerated sensitivity to UVR of melanocytes with loss-of-function MC1R compared to melanocytes with functional MC1R and comparable levels of melanin [93].

Exposure of NHM to UV results in the immediate formation of hydrogen peroxide and a dose-dependent generation of DNA photoproducts, which lead to the arrest of melanocytes in the G1 phase of the cell cycle and then to apoptosis. Treatment of melanocytes with melanocortins or ET-1 partially rescues them from the UV-induced G1 arrest [155]. The levels of cyclobutane dimers formed immediately following UVB was similar in melanocytes homozygous for R160W mutation in MC1R and in melanocytes with comparable melanin content and functional MC1R. The increased apoptosis observed in melanocytes with loss of function MC1R is due to the accumulation of DNA lesions and inefficient repair of UV-induced DNA damage. αMSH and ET-1 inhibit the apoptosis of melanocytes following UV exposure. Importantly, αMSH enhances the survival of melanocytes carrying functional MC1R, but not melanocytes expressing loss-of-function mutations in the *MC1R* gene (unpublished data). Comparison of functional and non-functional MC1R melanocytes suggests that a functional MC1R is necessary to cope with the DNA damage induced by UVR.

10. Indirect effects

Interestingly, UV has been shown to increase POMC production by keratinocytes [156]. It should be mentioned that in human epidermis the major POMC peptide seems to be ACTH [144], which is actually more efficient at activating MC1R than are the various forms of αMSH. Additionally, studies on murine melanoma cells have demonstrated that UVB radiation up-regulates the expression of MC1R [157]. Very recently, it has been shown that UV down-regulates the expression of neprilysine, a peptidase that cleaves and inactivates αMSH and ACTH [158]. Thus, UVR can modulate POMC action in the epidermis in three different ways that converge to reinforce their melanogenic functions. Effects of cAMP on melanocytes have been recently reviewed [159]. Melanocytes cultivated in

keratinocyte-conditioned media respond by increasing their growth, melanogenesis and dendricity [160]. These effects are enhanced by keratinocytes exposed to UVR, strongly suggesting that keratinocytes secrete specific factors responsible for melanocyte activation. Therefore, keratinocytes seem to be a key cellular component in the physiological tanning response [161]. Keratinocyte-secreted factors, which induce melanocyte activation, include prostaglandin PGE2 [162], αMSH and ACTH [156,163], endothelin-1 [164,165] and NO [160]. αMSH, ACTH and PGE2 activate the cAMP pathway in melanocytes, while NO activates the cGMP-dependent signaling events.

11. Melanocyte survival

In vitro, melanocytes respond to UV with dose-dependent growth arrest and reduction in survival [143,155,166]. Melanocytes have low proliferation capacity and it is reasonable to assume that due to their significance in photoprotection of the skin, mechanisms were selected insuring melanocyte survival. In fact, melanocytes are thought to resist apoptosis by constitutive expression of the anti-apoptotic protein Bcl2.

Many of the effects of UV on human skin are indirectly mediated by up-regulating the synthesis of various growth factors and cytokines, some of which function as paracrine or autocrine regulators of melanocytes [140,167]. Among those factors are αMSH and endothelin-1 (ET-1), synthesized by various epidermal cell types. αMSH and ET-1 are mitogenic and melanogenic to human melanocytes, and both are important participants in the melanogenic response of melanocytes to UV [155,166,168]. Recently, new roles for αMSH and ET-1 as pro-survival agents were described for UV-irradiated melanocytes. The survival effects of αMSH and ET-1 were shown to be independent of their melanogenic or mitogenic effects and to involve the activation of a critical survival pathway involving IP3 kinase and its substrate Akt/PKB [40].

The IP3 kinase pathway is an important survival pathway in many types of cells, such as neurons, fibroblasts and keratinocytes, and mediates the effects of various survival factors, such as insulin-like growth factor, nerve growth factor, platelet-derived growth factor, mast cell growth factor, epidermal growth factor and hepatocyte growth factor [169–174]. Activation of IP3 kinase results in increased levels of PIP3 and inositol 3,4-biphosphate, which ultimately lead to the activation of the serine–threonine kinase Akt/PKB. Activated Akt inhibits apoptosis, particularly the intrinsic apoptotic pathway, by phosphorylating and inactivating the pro-apoptotic proteins Bad and caspase 9, and additionally, Akt phosphorylates IκB, resulting in the activation of NFκB that inhibits apoptosis [175].

The cAMP dependent pathway, the main signaling pathway activated by αMSH through the MC1R, also stimulates Akt/PKB activity in an IP3K-independent mechanism [176]. Activation of the MAP kinases ERK1/2, and subsequently the transcription factor CREB, results in the phosphorylation and activation of Mitf in melanocytes, as demonstrated in melanocytes treated with stem cell factor, the c-kit ligand [177]. Treatment of human melanocytes with ET-1 activates the same MAP kinase pathway and phosphorylates CREB on serine 133; these effects are enhanced in the concomitant presence of αMSH [178,147]. Irradiation of human melanocytes with UV leads to the phosphorylation, and hence the activation, of Mitf which is downstream from CREB. ET-1 or αMSH also stimulate the phosphorylation of Mitf, and this effect might be regulated indirectly by Akt. While UV inhibits the level of Bcl2, treatment with ET-1 or αMSH markedly abrogated this effect.

Since UV-induced apoptosis occurs in cells with extensive DNA damage, it is plausible that the anti-apoptotic effect of αMSH is due to enhanced DNA repair, which would reduce UV induced mutagenesis and carcinogenesis.

12. UV-induced DNA damage

The most drastic effects of UV exposure are photoaging and photocarcinogenesis. Melanin guards against the photodamaging effects of UV by acting as a filter that limits the penetration of UV rays through the epidermis [90,91]. Melanin also acts as a scavenger of UV-induced reactive oxygen species that cause lipid peroxidation and damage proteins and DNA [179]. Acute exposure of the skin to solar UV results in ex-

tensive DNA damage, leading to apoptosis, which is best evidenced by the appearance of sunburn cells, i.e., apoptotic keratinocytes, in the epidermis [180]. UVB is the most cytotoxic and mutagenic waveband of solar radiation. UVB irradiation of mammalian cells produces damage at the level of proteins, lipids and DNA, according to the wavelengths that reach the cells. Many of the deleterious effects of UVB will occur in the DNA, since DNA bases directly absorb incident photons within this narrow wavelength range. UVB and UVC irradiation can generate pyrimidine dimer photoproducts, particularly cyclobutane pyrimidine dimers (CPD) and 6,4-photoproducts (64PP) (Fig. 3), at a ratio that varies from 4:1 to 10:1 [181,182]. The presence of these DNA lesions can potentially induce mutations and eventually lead to the development of skin cancer. The phototoxic effect of UVA radiation is much lower than UVB, since DNA is not a chromophore for the long UVA wavelengths [183]. However, UVA can produce DNA damage indirectly through the generation of oxidative stress.

Electron transfer or singlet molecular oxygen produced by UVB and UVA radiation targets the DNA base guanine, giving rise to 8-hydroxydeoxyguanosine (8-OHdG) in the DNA strand [184]. 8-OHdG is a miscoding lesion caused by G to T transversion that is

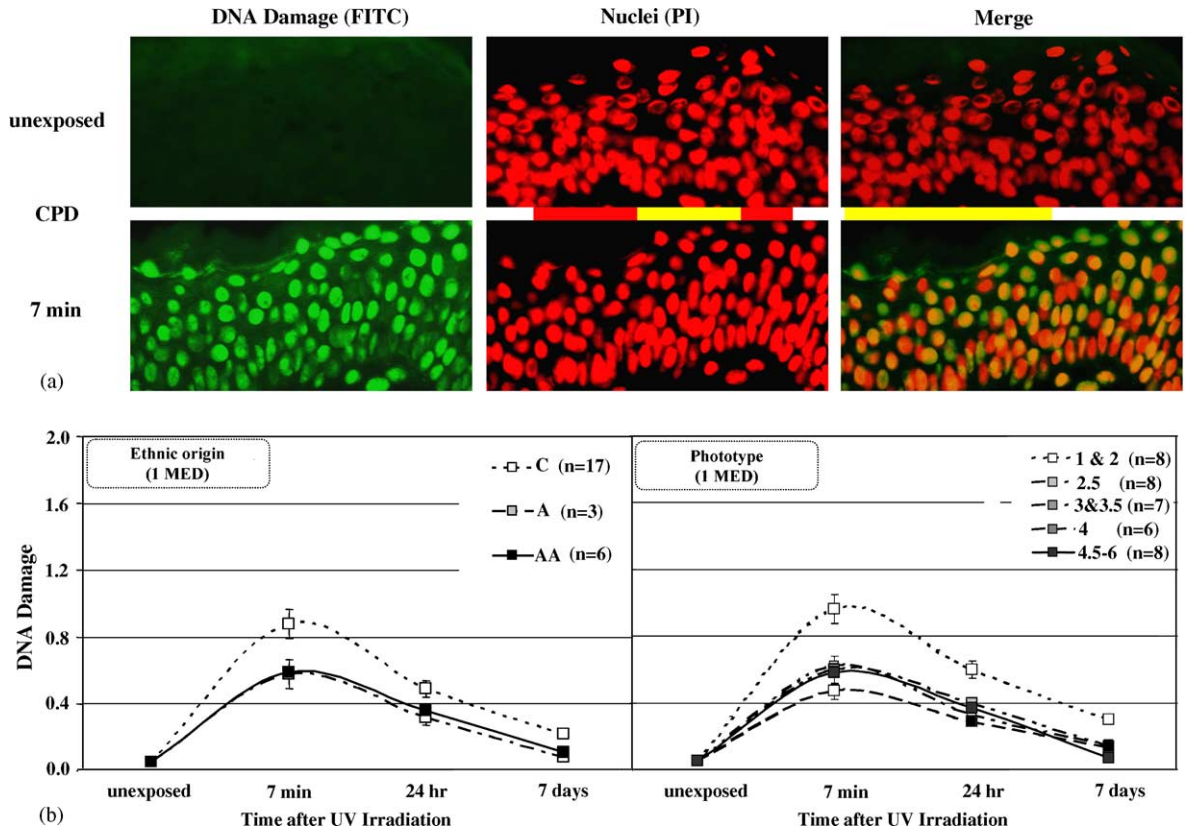

Fig. 3. (a) Immunohistochemical detection of CPD DNA photoproducts. DNA damage detected by FITC (green, left column) and nuclei detected by PI (red, central column) in UV-irradiated skin. Typical fluorescence image from one subject (S26, a Caucasian male, SPT 4.5) is shown. The right column shows the merged images, and colocalization of DNA over the nuclei is seen as the yellow color. Row 1 shows skin before UV irradiation and row 2 shows skin 7 min after UV exposure. Modified from Tadokoro et al., UV-induced DNA damage and melanin content in human skin differing in racial/ethnic origin. Taken from ref. [182]. (b) Effects of different skin characteristics on DNA damage. DNA damage (as CPD) was measured before 1 MED UV irradiation and various times thereafter. The data are grouped by ethnic origin (C: Caucasian, A: Asian, AA: African–American) and by SPT. Results are reported as means of each group and the error bars reflect the SE. UV-induced DNA damage and melanin content in human skin differing in racial/ethnic origin. Adapted in part from ref. [182].

known to be a ubiquitous marker of oxidative stress, since it can be generated under various agents such as peroxynitrite, OH radical and singlet oxygen [185]. Recently, it was demonstrated that UVA also induces DNA photoproducts [186].

UV-induced CPD and 64PP generate abnormalities in DNA structure that can be recognized and corrected by the highly conserved nucleotide excision repair (NER) system [187]. These bulky DNA lesions inhibit RNA polymerase II giving rise to a signal that results in a preferential repair of lesions located on transcribed strands. During the course of repair a 26–30 base oligonucleotide containing the lesion, the gap is filled by unscheduled DNA synthesis carried out by DNA polymerase in the presence of PCNA and various replication factors. This transcription-coupled repair is vital for cell survival after UV exposure. Its impairment gives rise to serious DNA repair deficiencies that are well documented by the high incidence of skin cancer on sun-exposed areas in xeroderma pigmentosum patients [188].

Base changes, such as 8-OHdG, that represent only a minor fraction of UV-induced mutations [185] are repaired by the base excision repair system [187]. NER is the main mechanism for repairing UVB-induced DNA damage in mammalian cells. Recently, the immunomodulatory mediator IL-12 has been shown to protect cells from apoptosis induced by DNA damaging UVB radiation, through the enhancement of NER activity [189]. A similar finding was demonstrated by the hepatocyte growth factor/scatter factor enhancing the repair of DNA strand breaks in cell lines carrying p53 mutations [190]. These are very significant findings since they uncover the possible role of cytokines in modulation of NER.

The observation that melanocytes with high eumelanin to pheomelanin ratios and loss-of-function MC1R, are more sensitive to UVR-induced cytotoxicity than are wild-type melanocytes regardless of constitutive pigmentation [93], suggests that the inability of melanocytes to respond to αMSH reduces their defense mechanisms against UVR genotoxicity. Preliminary results comparing functional and non-functional MC1R melanocytes show that the non-functional MC1R cells have a very slow rate of CPD removal, suggesting the importance of MC1R/αMSH in DNA repair (unpublished results).

13. UVR induces local and systemic immunosuppression

UVB radiation is among the most ubiquitous agents in the environment, and humans are invariably exposed to it. UVR not only initiates and promotes the transformation of normal epidermal cells to cancer cells via dysregulation of intracellular signaling pathways and via its mutagenic effects on cellular DNA, but also by altering the host immunity by reducing its capability for surveillance against tumor or viral antigens. Since UVB radiation is almost completely absorbed within the epidermis, epidermal cells are considered to be the targets for UV effects. Dendritic Langerhans cells reside in the suprabasal layer of the epidermis and they play an essential role in immune responses initiated in the skin. Their function is to pick up antigens or haptens within the epidermal layer and then to migrate to draining lymph nodes where antigen presentation to specific T cells occurs [191]. In vitro exposure to a narrow band of UVB radiation (312 nm) induces a potent inhibition of human Langerhans cells antigen presenting function [192]. Both local and systemic UVB-induced immunosuppression have been related to deleterious effects of this radiation on epidermal cells. Based on studies performed mainly in the murine system, immunosuppression has been shown to result both from direct effects of UVB radiation on Langerhans cells antigen-presenting function [193] and from indirect effects mediated through keratinocyte-derived suppressive factors [194].

Elmets et al. [195] demonstrated in the early 1980s that low doses of UVB radiation to the skin inhibited its response to haptens painted on irradiated sites, although haptens painted on unirradiated skin induced a strong contact hypersensitivity (CHS). Yoshikawa et al. [196] showed that most skin cancer patients exposed to UVB failed to develop CHS to haptens, whereas about 60% of healthy subjects developed vigorous CHS. These subjects who failed to induce an immune reaction were called UV-susceptible and the others were called UV-resistant. It was postulated that UV-susceptibility might be a risk factor for the development of skin cancer. Fisher and Kripke [197] reported that skin tumors that developed in adult mice exposed to high doses of UVB radiation for prolonged periods of time were rejected when transplanted to syngeneic healthy mice, but continued to grow

when transplanted to mouse skin pre-exposed to UVB radiation.

UVR induces keratinocytes to release IL-10, which alters antigen presenting cell function by preferentially activating Th2 suppressor cells. Th2 cells mediate their suppressive effects by releasing cytokines such as IL-4 and IL-10 [198,199]. The effects of IL-10 on the immune response are counteracted by IL-12, which blocks the suppression of delayed-type hypersensitivity reactions observed in UV-induced suppressor T cells, has the ability to stimulate Th1 cells [197] and blocks the differentiation of Th2 cells [200].

14. Anti-inflammatory effect of αMSH, role of MC1R?

αMSH is one mediator capable of counteracting inflammation. MC1R, first demonstrated in melanoma tumor cell lines [201], is present on immunologically important cells such as macrophages, monocytes, dendritic cells and neutrophils [202–205].

MC1R expression has been shown to be upregulated not only in melanocytes, but also in monocytes in response to external stimuli such as UVB-radiation and retinoic acid [206]. Support for the anti-inflammatory effects of αMSH is found in studies using a mouse model of CHS and the induction of hapten-specific tolerance in mice [206]. The immunomodulatory action of αMSH includes both immunostimulatory and immunosuppressive effects [207]. Several studies have shown that αMSH antagonizes the effects of pro-inflammatory cytokines such as IL-1α, IL-1β, IL-6 and tumor necrosis factor-α, and induces the production of anti-inflammatory cytokines [208,209].

Besides its presence in the skin and in circulating blood, αMSH is widely distributed in various regions in the brain. Inflammation, known as a localized response to tissue injury, can also be observed in neurological disorders that are associated with infectious agents as well as in other disorders such as multiple sclerosis, Alzheimer's disease, stroke and neurodegenerative disorders. αMSH is also known to modulate CNS inflammation. It was shown that the neuropeptide inhibits the production of tumor necrosis factor-α in murine brain [210]. The mechanism by which αMSH induces the anti-inflammatory effects is not completely understood, but it seems that the induced effect is extremely rapid.

15. Concluding remarks

Cancer of the skin is the most common of all cancers. Melanoma accounts for about 4% of skin cancer cases, but it causes about 79% of skin cancer deaths. The number of new cases of melanoma in the United States is on the rise. The American Cancer Society estimates that in 2004 there will be 64,200 new cases of melanoma in this country and that about 8600 people will die of this disease. Australia, Northern European and other countries are also paying a huge price to skin cancer. There is an urgency to bring together an appropriate scientific task force to fight against what tomorrow could be a tremendous public health tragedy. We focus our efforts on characterizing the regulation of mammalian pigmentation, with the ultimate goal of optimizing skin pigmentation and photoprotection, and in developing novel approaches for targeting malignant melanoma. The importance of skin pigmentation, particularly with respect to photoprotection against skin cancers (including malignant melanoma), has underscored the importance of such research. The alarming increase in incidence of all types of skin cancers in recent decades has also been a major stimulus.

In this paper, we have reviewed advances of the role of the MC1R and the responses of melanocytes to UVR. MC1R functions as a primary regulator of eumelanin synthesis in human melanocytes, and therefore is the key control point of melanocyte responses to UVR. Given the significance of the MC1R in determining the risk for skin cancer, particularly melanoma, preventative strategies should be developed based on activating the receptor by potent melanocortin agonists. This would be of particular benefit to individuals that are heterozygous for one allelic variant of MC1R, with a reduced response to endogenous melanocortins and with a high risk for melanoma.

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